

## Microbial Synthesis of Iodophile Polysaccharide by a *Clostridium* from the Cæcum of the Pig

MICROSCOPIC findings of Baker and his collaborators<sup>1</sup> have repeatedly confirmed the observation of Henneberg<sup>2</sup> that the microbial decomposition of carbohydrates in the gastro-intestinal tract may be accompanied by extensive synthesis of iodophile material within the bacterial cell. Where, as in the rumen microflora of the ox<sup>3</sup>, biochemical observations have been attempted, they have shown that the iodophile material is a polysaccharide. A knowledge of the mechanism of such synthesis is essential, therefore, to a fuller understanding of the chemical changes accompanying the microbial breakdown of dietary carbohydrates, including starch and cellulose, in the rumen and cæcum of various animal species.

Although the taxonomic identity and growth requirements of many of the iodophile micro-organisms concerned are still unknown, in some instances they can be grown and identified in pure culture. Baker and Nasr<sup>4</sup>, and Baker, Nasr and Morrice<sup>5</sup> have shown that an iodophile strain of *Clostridium butyricum* is primarily responsible for the breakdown of raw potato starch in the cæcum of the pig. The organism was isolated in pure culture on modified Beijerinck's medium<sup>6</sup>. Washed suspensions were then prepared according to the technique employed by Hehre and Hamilton<sup>7</sup> and Hehre<sup>8</sup> in the investigation of polysaccharide synthesis in *Neisseria pharyngitidis*, and buffered with sodium citrate. Preparations were made from young cultures, which showed little or no iodine reaction, as well as from older cultures in which the micro-organisms were replete with iodine-reacting material.

To suspensions of young cultures, glucose, glucose-1-phosphate and sucrose, respectively, were added in 2 per cent concentrations. Fructose and raffinose were also included in experiments made on older cultures. The suspensions with the various sugars added, together with control suspensions made up with buffer alone, were incubated at 37° C. A distinct, though faint, macroscopic iodine reaction was visible in the centrifuged deposit from the glucose-1-phosphate suspensions of young cells after 20 hr. After three days a strong reaction was obtained. The macroscopic findings were confirmed by microscopic examination of the micro-organisms, the contents of which were deeply stained. No reaction, macroscopic or microscopic, was observed in the control, glucose or sucrose suspensions. These observations indicated that a phosphorylating mechanism was involved in the endocellular synthesis of polysaccharide by young cells. In the older cultures the initial iodine reaction was retained in the control and glucose-1-phosphate, but disappeared in the glucose, sucrose, fructose and raffinose suspensions. The reason for the difference in behaviour between glucose-1-phosphate and the other sugars requires further investigation.

In conclusion, it may be recalled that Hehre<sup>8</sup> demonstrated that washed suspensions of *Neisseria pharyngitidis* synthesized iodophile polysaccharide directly from sucrose. The mechanism of synthesis, therefore, requires individual investigation for each of the isolable members of a mixed iodophile population. Thus the complex microbial facies of the gastro-intestinal tract of men and animals affords a rich province, as yet only incompletely explored, for the investigation of polysaccharide synthesis in a variety of bacteria, yeasts and protozoa.

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<sup>1</sup> References 1933-47, Baker, F., and Harriss, S., *Nutr. Abstr. Rev.*, 17, 3 (1947-48).

<sup>2</sup> Henneberg, W., *Zbl. Bakt.*, Ab. II, 55, 242 (1922).

<sup>3</sup> Smith, J. A. B., and Baker, F., *Biochem. J.*, 38, 496 (1944).

<sup>4</sup> Baker, F., and Nasr, H., *J. Roy. Micro. Soc.*, 67, 27 (1947).

<sup>5</sup> Baker, F., Nasr, H., and Morrice, F., *J. Gen. Microbiol.*, 3 (No. 1), Proc. xv (1948).

<sup>6</sup> Percival, J., "Principles of Agricultural Microbiology" (Duckworth, London, 1922).

<sup>7</sup> Hehre, E. J., and Hamilton, D. M., *J. Biol. Chem.*, 166, No. 2, 777 (1946).

<sup>8</sup> Hehre, H., *J. Bact.*, 55, No. 2, 197 (1948).

## Participation of 'Brown Fat' Tissue in the Alarm Reaction

IN the course of our studies on the general-adaptation syndrome, we were surprised to note the intense morphological changes which occur, during the alarm reaction stage, in the so-called 'brown fat'. It will be recalled that in various animal species, a special type of adipose tissue occurs, which, upon naked-eye inspection, is characterized by its brownish colour. Histologically, it resembles a lipid-storing endocrine gland (such as the adrenal cortex, the corpus luteum or the Leydig cells of the testis), because its epithelioid cells contain numerous small lipid granules and are richly supplied with capillaries.

Such 'brown fat' (synonyms: primitive fat organ, fat gland, hibernating gland, interscapular gland, lipoid gland, cholesterol gland, glandula insularis cervicalis, multilocular adipose tissue) is normally present throughout life in certain species, such as most rodents and hibernating animals. In other species, including man, it occurs quite regularly in certain locations (for example, the vicinity of the parathyroids, thymus and kidney) during embryonic life, but later tends to become completely transformed into common adipose tissue.

In hibernating animals, the chief function of the organ appears to be the storage of certain lipids during the active season, in order to make them available throughout the hibernating period. The purpose of 'brown fat' in non-hibernating animals is rather problematical. Cramer<sup>1</sup> postulated a close relationship between the lipid content of this tissue and that of the adrenal cortex, emphasizing that both these organs are extremely rich in cholesterol. Sweet and Hoskins<sup>2</sup> found that the 'brown fat' of the wood-chuck contains as much testoid material (the equivalent of 100 γ of androsterone per 50 gm. of tissue) as bull testis, the richest natural source of testoids. Vignes<sup>3</sup> refuses to believe that the gland acts merely as a reserve of nutritive material, since in rats even mere extirpation of the interscapular accumulation of 'brown fat' causes emaciation and death.

An intense and rapid discharge of adrenal lipids is one of the salient manifestations of the alarm reaction<sup>4</sup>, while fat from common adipose cells is much more slowly lost under the influence of non-specific systemic stress. We now find that the discharge of adrenal cortical lipids parallels the loss of sudanophilic material from the 'brown fat'.

In a preliminary experiment, twelve adult female piebald rats (average body-weight 120 gm.) were

divided into three groups of four animals each. The first group served as untreated controls, the second was exposed to cold, but allowed food, and the third was fasted during exposure to cold (0 to +5° C. during 16 hr.). At autopsy the interscapular 'brown fat' of the untreated animals was yellowish-white and well developed, while that of the rats exposed to the stress of cold was partly involuted, brownish-red and extremely edematous. Gastric ulcers, thymico-lymphatic involution and marked discharge of adrenal cortical lipids showed that this degree of cold produced a severe alarm reaction, especially in the fasted animals.

Upon histological examination of the 'brown fat', it was noted that the sudanophilic granules were almost completely discharged in the animals submitted to cold and fasting, but only partially discharged in those exposed to cold while taking food.

In a second series of experiments, twenty adult male piebald rats (average body-weight 130 gm.) were subdivided into four groups of five animals each. Group I acted as untreated controls; group II was exposed to cold (0 to +5° C.); in group III the spinal cord was trans-sixed at the height of the seventh cervical vertebra, while the rats of group IV were repeatedly forced to exercise in a revolving cage until they collapsed from fatigue. Since recent experiments demonstrated that the phagocytic activity of reticuloendothelial macrophages

is greatly increased during the alarm reaction, we injected the animals of all four groups intravenously (one hour before they were to be killed) with 2 c.c. of a dilute solution of Higgins Indian ink.

The rats were killed twenty-four hours after initiation of the experiment. At that time the interscapular 'brown fat' showed marked oedema, hyperaemia, discharge of sudanophilic lipids and increased phagocytosis of Indian ink by the macrophages of this tissue. Simultaneously other manifestations of the alarm reaction were noted in all stressed groups, irrespective of the stimulus used.

It may be concluded that discharge of lipids from the brown fat represents a characteristic response during exposure to various types of systemic stress; it appears to be a sensitive early indicator of catabolism during the alarm reaction.

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<sup>1</sup> Cramer, W., *Brit. J. Exper. Pathol.*, 184 (1920).

<sup>2</sup> Sweet, J. E., and Hoskins, W. H., *Proc. Soc. Exper. Biol. and Med.*, 45, 60 (1940).

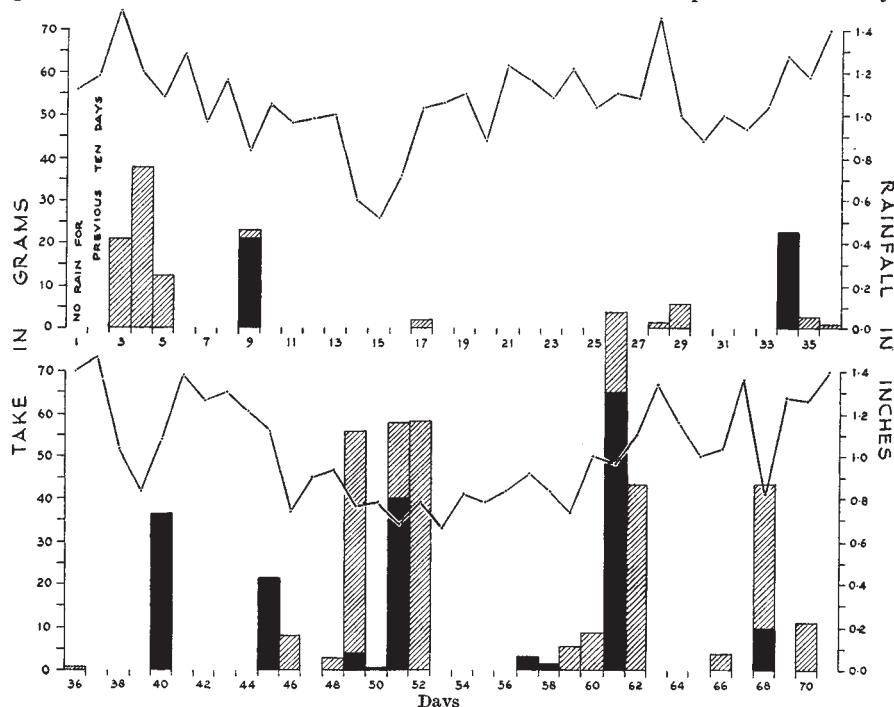
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### Effect of Rain on the Feeding of the Malaysian Rice-field Rat

*Rattus rattus argentiventer*, the Malaysian rice-field rat, is a common pest of standing crops and of wasteland covered by the tall grass 'lalang' (*Imperata cylindrica*). It is widely distributed throughout the Malaysian Islands, and in Malaya it is the most important host of the scrub typhus mite-vector *Trombicula akamushi* s.l.

During feeding tests on this rat, it was found that the amount of foodstuff taken from covered baiting points was related to the rainfall. In this part of Malaya rain falls intermittently in heavy showers, and it was found that rain after a period of some days



of drought was usually followed by a conspicuous increase in the food taken, while rain during the night tended to cause a decrease.

Tests were carried out in an area of lalang-covered wasteland. Dry rice was offered in covered boxes of a type which will be described elsewhere. Five baiting points were laid and weighed daily; but only the total take is recorded here. Rainfall was measured on a self-recording gauge. Rain charts and baits were changed daily at about 9 a.m., so the 'day' in the results quoted runs from 9 a.m. to 9 a.m.

The accompanying figure shows the bait-take and rainfall daily for 71 days. The rainfall blocks are shaded thinly for rain during daylight and solid for rain during the night.

It will be seen that on days 3, 9, 17, 28, 34, 40, 45, 57 and 66, each after three or more rainless days, an increase in take is shown on seven occasions and a decrease on two only. Such a result would be expected by chance about once in eleven trials. Further scrutiny, however, reveals that on days 9 and 45, when the take decreased, there was rain at night lasting on each occasion for some hours; whereas on days 34, 40 and 57, when rain fell at night also but the take increased, the shower lasted less than an hour on each occasion.